REMARKS

The Examiner has found the oath or declaration to be defective. A new oath or declaration will be filed.

Claims 1 – 6, 8 – 29, and 31 - 33 are pending in the application. Claim 5 is objected to due to a period after the word "electrical." The period has been deleted from claim 5, as reflected in the amended claims. Claims 1 – 6, 8 – 29, and 31 – 33 have been rejected under 35 U.S.C. §112, first paragraph. Claims 1 and 17 have been rejected under 35 U.S.C. § 102(b); claims 1 – 2, 8 – 12, 15 – 19, and 26 – 29 have been rejected under 35 U.S.C. § 102(e). Claims 23 – 24 have been cancelled. Claims 15 and 16 have been amended as discussed with Examiner McGillem. No new matter has been added.

35 U.S.C. § 112 Rejections

Claims 1 – 6, 8 – 29, and 31 – 33 have been rejected under 35 U.S.C. §112, first paragraph. The Examiner alleges that the specification, while being enabling for *in vitro* selective electrofusion of at least two fusion partners having cell-like membranes, does not reasonably provide enablement for *in vivo* electrofusion of at least two fusion partners, or for conducting *in vitro* fertilization, or for conducting non-human cloning. Claims 23-24, directed to *in vitro* fertilization and cloning have been cancelled. Applicants respectfully traverse the rejection.

The instant method is directed to highly focused electrofusion between at least two fusion partners having cell-like membranes. The teaching of the specification, including the examples provided and combined with what is known in the art, provides ample support for using the method of the invention in *in vivo* electrofusion of at least two fusion partners and enables one skilled in the art to make and use the instant invention without undue experimentation.

Electrofusion involves the application of an electrical field over phopholipid bilayer membranes to induce pore formation. The specification contemplates use of the invention in a number of *in vitro* and *in vivo* settings, and teaches the variety of biological experiments in which electrofusion has been used, including *in vivo* methods of fusion [see, e.g. Ogura A., et al.,

1995, Reprod. Fertil. Dev; Van Stekelenberg-Hamers, A.E.P. et al., Mol. Reprod. Dev. 1993; Heller, R. et al., Biochim. Biophys. Acta. 1990]. For example, Applicants provide numerous other examples in the background of the invention section of the specification detailing extensive list of references detailing the use of electrofusion in a variety of biological settings. In addition, the specification specifically details the use of the method for *in vivo* fusion of cells and other fusion partners (see, for example, pages 11-13 and Example 3), and provides a an example of a clinical application of the method according to the invention (see, for example, pate 5 and Figure 5/5).

The Examiner alleges that the state of the art is unpredictable; however electroporation is well-known in the art, and has well-described uses in a number of different methods (see references from the Background of the Invention, as described above). The Examiner points to Sakai et al. as an example of the unpredictability of *in vitro* reproductive techniques. The instant method provides improvement over bulk electrofusion techniques in offering control over the fusion process. The method provides a highly focused electrical field allowing the precision to fuse even a single pair of cells. Supporting our argument, the Examiner points out that Sakai et al teaches a shortcoming of bulk electrofusion is the low proportion of live offspring (see, for example, page 4 of the Office Action). The instant Application teaches use of the method to overcome the shortcomings of bulk electrofusion by offering complete over the fusion process, obviating doubts as to the identity of the somatic cells. Thus, the instant method is highly useful in procedures such as *in vitro* fertilization and cloning, where precise fusion is desirable.

The Examiner also points to Orentas et al., which uses PEG based methods and to Mekid and Mir who acknowledge the shortcomings of Electrofusion. In contract, Applicants teach and fully enable a highly focused method of electrofusion to overcome this shortcoming.

The Examiner alleges that the invention is unpredictable because the instant method of electrofusion "might result in uneven fusion among adjacent cells wherein one cell may fuse with many liposomes." (See page 8 of the Office Acion). As discussed in the Specification, methods of bulk electrofusion do not allow control over the number of cells to be fused together, and often results in unwanted fusion between cells (see, for example pages 2 – 3). The instant invention overcomes the problems of bulk electrofusion by providing a method for electrofusion of single cells, thus allowing for fusion between even a single pair of cells. Applicants point out,

for example, in Figure 2, page 4, and Figure 2/5 of the specification that the claimed method allows for the precise fusion between two fusion partners. Figure 2 A-C shows the alignment of a liposome with a selected cell using a microelectrode, and Figure 2D-E shows six flourescein containing liposomes brought in to contact with a cell. Figure 2G shows that no resulting background fluorescence is detected, thus illustrating that electropermeabilization of the liposome, and electrofusion between the selected cell and liposome using a microelectrode as illustrated in the figure, is possible. Figure 3 , pages 4 – 5 and Fig. 3/5 shows a cell-cell fusion sequence using the method of the invention, and Figure 4, page 5 and Fig. 4/5 is a series of bright-field images that show electrofusion between two cells. Together, these examples illustrate successful fusion of two fusion partners using the claimed method.

The Examiner alleges that the amount of guidance provided does not enable the invention as claimed. The invention relates to a method for selective electrofusion of at least two fusion partners having cell-like membranes. The disclosure and examples provide ample guidance for use of the method for the fusion of two fusion partners having cell-like membranes, as discussed above. As taught in the specification, included in a fusion partner having a cell-like membrane is a single cell, a liposome, a proteoliposome, a synthetic vesicle, a plant protoplast, an egg cell, a sperm or spermatid, and an enucleated egg cell (see for example, page 6). The specification teaches manipulation and alignment of the fusion partners, and provides examples demonstrating methods that have been used to manipulate organelles of small dimension (see for example, page 7). The specification teaches the use of a micropositioner to position the cells. Specifically, the specification teaches micromanipulation and fusion (see for example, page 13), an experimental protocol including optical trapping (p.14), the instrumentation used for electrofusion (see for example, page 15), and micromanipulation using microelectrodes (see for example, page 17).

The specification provides ample guidance as to how to obtain an electrical field by use of a low or high voltage pulse generator sufficient to result in fusion between two fusion partners, as taught in methods of the invention. The specification teaches a range and duration of voltages used in the method, as well as the voltage that should be measured at the membrane of the fusion partners, and gives examples of pulse repetition rates that are suitable for use in the claimed method (see for example, page 8, lines 8-29). Further, the specification discloses the preferred distance to position the microelectrodes from the fusion partners in order to provide a

highly focused electrical field. The specification teaches the type of electrode to use and their preferred diameters (see for example, page 9). Figure 1 illustrates in detail the experimental set up and how it is used in the method (see for example, page 4, 10 and Fig. 1/5), and Figure 5 provides an example of an *in vivo* set up (see for example, page 5 and Fig. 5/5). Example 1 provides a detailed description of cell-cell fusion, and indicates the voltage and number of pulses used, as well as provides the type of buffer preferred for successful fusion of two fusion partners (see for example, page 18 – 19 and Figs. 3 A and 3B). Example 2 (pages 20 – 21) illustrates use of the method in cell-liposome fusion, and provides the conditions that were used for successful fusion (see, for example p.21, lines 6 – 20). Example 3 teaches use of single open-bore silicon capillaries, setup according to Figure 1, and positioned using micromanipulators to fuse two cells. Figure 4 shows that this set-up successfully allows for electrofusion between two cells.

The disclosure and examples provide ample evidence for the successful fusion of two fusion partners using the method of the invention. Accordingly, Applicants request withdrawal of the rejection and allowance of the claims.

35 U.S.C. § 102(b) Rejection

Claims 1 and 17 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Magae et al. (Appl. Micro. Biotechol., 1986, Vol 24, 509-511.) The Examiner alleges that the Magae et al. reference teaches "a method to fuse two giant plant protoplasts by using two glass electrodes prepared from glass capillaries and attached to a micromanipulator." Applicants respectfully submit that the invention as claimed is not anticipated by the Magae et al. reference, and respectfully traverse the rejection.

The instant invention is directed to a method for selective electrofusion of at least two fusion partners having cell-like membranes, the method providing a highly focused electric field using at least one microelectrode that is positioned by use of a microscope, at least one micropositioner and/or a stereotactic device, and wherein at least one microelectrode is hollow, and sufficiently small to permit the selective fusion of two fusion partners.

Magae et al. teach a method to fuse two giant plant protoplasts using glass electrodes and attached to a micromanipulator. The instant invention is distinguished over the prior art in using at least a single electrode to provide a highly focused electric field for the fusion of at least two fusion partners as defined by the invention. The instant invention is based on a method that

allows controllable fusion of single cells. The prior art objection alleged by the Examiner does not address these aspects of the instant invention. Magae et al. do not provide a method of selective electrofusion that comprises bringing the fusion partners in to contact and applying a highly focused electric field. The method of Magae et al. is a bulk electrofusion, made more efficient by manipulation of the conditions and size of the cell. Thus, the claims are not anticipated by Makae et al.

As such, the teaching of the Magae et al. reference do not anticipate the instant methods of selective electrofusion of at least two fusion partners having cell-like membranes using a highly focused electric field. Accordingly, Applicants respectfully request the withdrawal of the rejections and allowance of the claims.

35 U.S.C. § 102(e) Rejection

Claims 1 – 2, 8 – 12, 15 – 19, and 26 – 29 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Pui et al, US Patent No. 6,093,557 (the '557 patent). The Examiner alleges that the '557 patent discloses, "a method and apparatus for fusion of cells with vesicles or liposomes that comprises a capillary electrode through which the liposomes are dispersed in a spray on to target cells." Applicants respectfully submit that the invention as claimed is not anticipated by the '557 patent and respectfully traverse the rejection.

The claimed invention is directed to a method for selective electrofusion of at least two fusion partners having cell-like membranes, the method providing a highly focused electric field using at least one microelectrode that is positioned by use of a microscope, at least one micropositioner and/or a stereotactic device, and wherein at least one microelectrode is hollow, and sufficiently small to permit the selective fusion of two fusion partners. The '557 patent does not teach or suggest a method that provides a highly focused electric field and allows for the selective fusion of two fusion partners. Rather, the '557 patent is drawn to a method for introducing biological material into cells that depends on establishing a spray of electrically charged, dispersed particles. According to the '557 invention, the electrical charge of the dispersed particles is used to provide one or more of the dispersed particles with a velocity sufficient for the introduction of one or more substantially dispersed particles into one or more of the target cells. The i '557 patent thus depends on acceleration of the particles such that some

particles will obtain a velocity sufficient to enable them to penetrate cells. The '557 invention uses a spray established in the region of a target including one or more cells (Figure 1A description). The spray of charged particles are not an electric field that is highly focused on the fusion partners. The '557 reference nowhere contains a reference to a highly focused electric field, nor does it mention selectivity of fusion between two fusion partners.

As such, the teaching of the '557patent does not anticipate the currently pending claims. Accordingly, the '557 patent does not anticipate the instant claims and Applicants respectfully request the withdrawal of the rejections and allowance of the claims.

Applicants request the withdrawal of the rejection and allowance of the claims.

CONCLUSION

In light of the above remarks, Applicants respectfully requests early consideration and allowance of the subject application.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned attorney would appreciate the opportunity to do so.

Respectfully submitted,

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